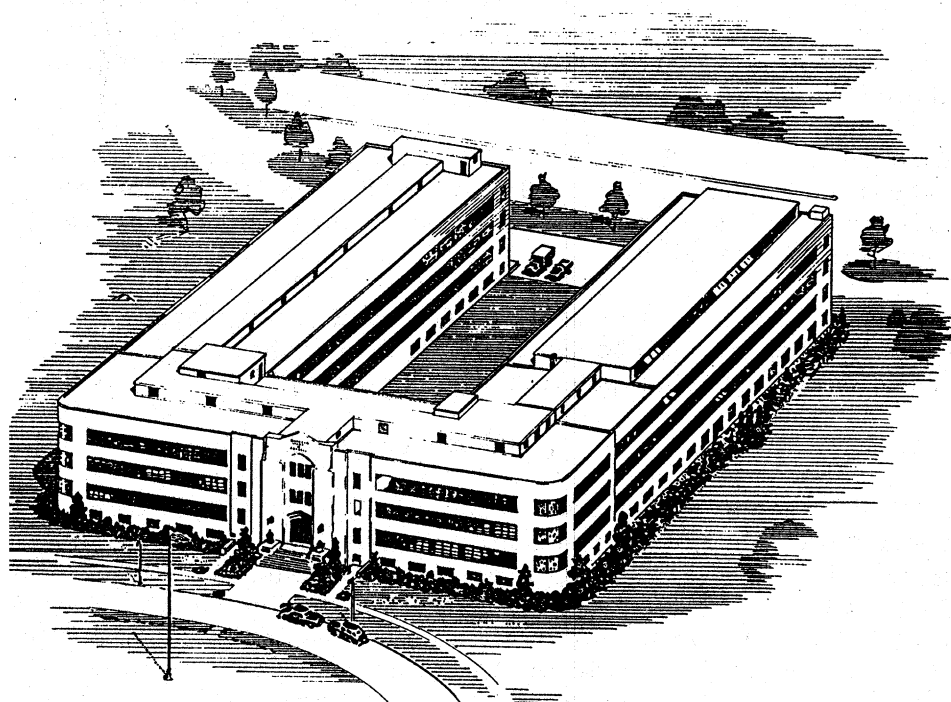


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AGAINST MOSAIC DISEASES

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PRELIMINARY STUDY OF STERILIZING TOBACCO STEMS AGAINST MOSAIC DISEASES

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The supply of tobacco stems has been considerably augmented in recent years by the increased consumption of cigarettes and by the decreased use of the stems for the preparation of nicotine insecticides. These stems could be used directly as fertilizer if it were not for the danger of spreading mosaic diseases. The residual stems from nicotine manufacture, which are sterile because of the lime and heat used in the process (2), are highly regarded as a fertilizer and as a conditioner in mixed fertilizers (3). It has been shown (1) that infected tobacco stems and refuse used as a fertilizer for tobacco land may infect the new crop. Some of the diseases carried by tobacco stems are: tobacco mosaic virus, black shank,³ bacterial (Granville) wilt⁴ and wild fire⁵. The wide distribution of these diseases in tobacco-growing areas makes it imperative that measures be taken to prevent their spread.

References in the literature (4,5) and correspondence with leading workers in the field revealed that little work has been done on the viability of mosaic in cured tobacco and its stems. Nearly all mosaic studies have concerned the living plant.

The present paper is the report of a preliminary study on the conditions required to sterilize tobacco stems. Since the work cannot be continued, it is deemed advisable to publish the present data.

Methods and Materials

The three factors studied were the effects of temperature, time of exposure, and moisture content of the stems on the mosaic virus. A fourth factor, the size of the treated pieces, should be considered. Bawden (6) reported that heating at 92° C. (197.6° F.) for 10 minutes inactivated mosaic virus *in vivo*. Since the fibrous stem of cured tobacco offers more resistance to heat than the green living stem, virus inactivation in cured tobacco is more difficult and would require more drastic treatment.

Leaf tobacco having about 60 percent mosaic infection was taken from the tobacco plots at the University of Maryland Tobacco Research Farm, Upper Marlboro, Maryland, in 1950. It was stemmed in the usual commercial manner. The broken stems, which ranged from 1 to 3 inches in length, were thoroughly

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³ *Phytophthora parasitica* (DAST.) VAR. *nicotianae* (BREA DE HAAN) TUCKER.

⁴ *Pseudomonas solanacearum* E. F. SMITH.

⁵ *Pseudomonas tobaci* (WOLF AND FOSTER) F. L. STEVENS.

mixed in a Robinson blender. Four samples were drawn. The first contained 32 percent moisture, the same as the original lot received from the factory. Three other samples were dried at 100° F. to adjust their moisture contents to 24, 16, and 8 percent, respectively. All samples were then stored in closed containers. A moisture content of 32 percent was considered typical of freshly stripped cigar tobacco stems; 24 percent and 16 percent as the average range of Burley and flue-cured stems; and 8 percent as representative of ordinary dry stems.

The temperatures used were 170°, 190°, and 212° F. Each group of stems, classified according to moisture content, was heated for 5-, 15-, and 30-minute periods; a total of 9 treatments on 36 samples. Unheated samples from each of the groups, and untreated samples from the original factory lot were included as checks. Attempts were made to maintain a high humidity in the dryer during the treatments.

An experimental dryer developed at the Eastern Regional Research Laboratory (7) was used for heating the material. In this dryer, heated air is forced at high velocity through a porous bed of stem material in a metal tray with a perforated bottom. Temperature readings were taken at three points in the apparatus, and on the top and in the middle of the mat of stems. Two-pound samples were used in the dryer.

After treatment, each sample was thoroughly dried at 110° F. in a tray dryer, ground in a Wiley mill through a 1-mm. screen, and then thoroughly mixed. To prevent contamination of one sample with dust particles of another, the Wiley mill was carefully cleaned after each sample was ground. Dust was removed by an air blast. Then all parts of the mill were treated with a soap solution, a formaldehyde bath, a steam blast, and an alcohol wash. Sanitary procedures were used throughout.

All glassware and inoculating equipment used in the tests were sterilized at 250° F. and 15 pounds pressure for 30 minutes. Ten milliliters of tobacco dust was used for each sample. The weight of the 10-ml. samples ranged from 6.3 to 7.6 grams. Thirty-five ml. of sodium phosphate buffer solution, pH 7.2, was added to each sample, except 14, 15, and 16 (Table 1). The buffer used at this pH extracted more of the virus than if water was used. Samples 14, 15, and 16 required 40 ml. of buffer to wet the material thoroughly. Most of the samples varied in the amount of buffer they would absorb. The amount of buffer added was enough to wet the material and allow a few milliliters for inoculating the test plants, *Nicotiana glutinosa*. The samples were allowed to soak in the buffer solution, with occasional stirring, for 4 hours before the plants were inoculated.

Inoculations were made by dipping the bent end of a glass rod wrapped with fine cheesecloth in the sample, and then rubbing it gently over the entire upper leaf surface, which had previously been dusted with carborundum (8). Except for the use of carborundum and the bent rod, the inoculation procedures were the same as those developed by Holmes (9). After the inoculation, the excess carborundum was washed off, and the plants were heavily fertilized with phosphate and nitrogen and removed to the greenhouse. Local lesions were counted one week after the inoculations were made (Table 1).

TABLE 1. HEAT INACTIVATION OF TOBACCO MOSAIC IN STEMS OF CURED TOBACCO WITH DIFFERENT MOISTURE CONTENTS, TEMPERATURES, AND PERIODS OF EXPOSURE TO HEAT. LOCAL LESION COUNTS ON *NICOTIANA GLUTINOSA*.

Percent moisture content	Heated for 5 min.			Heated for 15 min.			Heated for 30 min.			Unheated checks for each percent moisture content Heated for 60 min.		
	Sample	pH	Lesions ¹	Sample	pH	Lesions	Sample	pH	Lesions	Sample	pH	Lesions
	170° F.											
8	1	7.50	10.5	2	7.42	0.0	3	7.20	6.0	42	7.52	13.0
16.	4	7.40	6.5	5	7.38	15.0	6	7.35	1.0	43	7.46	13.0
24	7	7.40	5.0	8	7.30	4.0	9	7.22	1.5	44	7.55	50.5
32	10	7.55	1.0	11	7.50	2.5	12	7.40	0.0	45	9.10	11.0
190° F.												
8	13	7.60	25.5	14	7.62	6.5	15	7.57	5.5			
16.	16.	7.60	0.0	17	7.62	0.0	18	7.25	0.0			
24	19	7.55	0.0	20	7.55	0.0	21	7.40	0.0			
32	22	7.50	29.5	23	7.30	14.0	24	7.20	0.5			
212° F.												
8	25	7.60	1.0	26.	7.50	1.0	27	7.50	0.0	38	7.60	0.0
16.	28	7.50	13.0	29	7.20	3.5	30	6.90	4.0			
24	31	7.20	3.0	32	7.00	0.5	33	7.05	0.0			
32	34	8.40	1.0	35	8.50	0.0	36.	8.20	0.0			
32							37	8.88	0.0			
Autoclaved at 218.5° F.												
39		7.30	7.5	40	7.70	3.5	41	7.70	0.0			

Untreated samples (including checks)

Sample	pH	Lesions
1	7.2	1
2	7.2	1
3	7.2	1
4	7.2	1
5	7.2	1
6	7.2	1
7	7.2	1
8	7.2	1
9	7.2	1
10	7.2	1
11	7.2	1
12	7.2	1
13	7.2	1
14	7.2	1
15	7.2	1
16	7.2	1
17	7.2	1
18	7.2	1
19	7.2	1
20	7.2	1
21	7.2	1
22	7.2	1
23	7.2	1
24	7.2	1
25	7.2	1
26	7.2	1
27	7.2	1
28	7.2	1
29	7.2	1
30	7.2	1
31	7.2	1
32	7.2	1
33	7.2	1
34	7.2	1
35	7.2	1
36	7.2	1
37	7.2	1
38	7.2	1
39	7.2	1
40	7.2	1
41	7.2	1
42	7.2	1
43	7.2	1
44	7.2	1
45	7.2	1
46	7.2	1
47	7.2	1
48	7.2	1
49	7.2	1
50	7.2	1
51	7.2	1
52	7.2	1
53	7.2	1
54	7.2	1
55	7.2	1
56	7.2	1
57	7.2	1
58	7.2	1
59	7.2	1
60	7.2	1
61	7.2	1
62	7.2	1
63	7.2	1
64	7.2	1
65	7.2	1
66	7.2	1
67	7.2	1
68	7.2	1
69	7.2	1
70	7.2	1
71	7.2	1
72	7.2	1
73	7.2	1
74	7.2	1
75	7.2	1
76	7.2	1
77	7.2	1
78	7.2	1
79	7.2	1
80	7.2	1
81	7.2	1
82	7.2	1
83	7.2	1
84	7.2	1
85	7.2	1
86	7.2	1
87	7.2	1
88	7.2	1
89	7.2	1
90	7.2	1
91	7.2	1
92	7.2	1
93	7.2	1
94	7.2	1
95	7.2	1
96	7.2	1
97	7.2	1
98	7.2	1
99	7.2	1
100	7.2	1

Blended sample check of above lots from factory; 60 percent infection.
Stemmary sample check of above lots from factory; 60 percent infection.
Cured Burley stems, 1948 crop, percent infection unknown.
Cured Pennsylvania stems, 1948 crop; percent infection unknown.
Flue-cured stems; percent infection unknown.
Maryland leaf web; 60 percent infection.
Fresh green stem material.

¹ Average lesions from 2 plants (5 whole leaves per plant).

The tobacco mosaic virus tests were made in a greenhouse at the University of Maryland, College Park, Maryland. The test plants, *Nicotiana glutinosa*, were 13 weeks old; all had 5 to 10 leaves that could be inoculated (9). The plants were placed in the dark in a constant-temperature chamber at 80° F. for 48 hours (6), and then inoculated at night when the temperature was between 75 and 78° F. Five successive leaves on each test plant were used in the inoculation tests (9). Two plants were used for testing each sample.

Results

The pertinent data are summarized in Table 1. The results are rather erratic, probably because of the inherent difficulty in sampling and testing for the virus. The table shows that the virus was inactivated at 212° F. in the 32 percent moisture sample in 15 and 30 minutes' exposure (samples 35 and 36) and in the 24 percent moisture sample in 30 minutes (sample 33). This difference in the two lots of stems indicates that the moisture content of the stems is directly correlated with heat penetration into the material. The higher the moisture content of the stems, the more rapid and thorough is the heat penetration. The virus in the 32 percent moisture sample was not inactivated in 5 minutes at 212° F. The virus in the 16 percent moisture stem was not inactivated in 30 minutes at 212° F. Samples 2 and 12 -- 8 and 32 percent moisture samples -- heated at 170° F. showed no virus. Tests were repeated three times. Negative results obtained in samples 16, 17, 18, 19, 20, and 21 are hard to explain, in view of the fact that positive results were obtained in both the 8 and 32 percent moisture samples for each time exposed at 190° F. Also, there were positive results in the 212° F. tests. Since the entire procedure of treatment and inoculation cannot be repeated, it is impossible to determine the point at which the discrepancies occurred.

Comparison of the number of lesions in the fresh green plant material of sample 52 with the number of lesions in sample 51 and the two check samples 46 and 47 gives some indication of the amount of inactivation of the virus in dry cured material.

The wide range in the number of lesions in the unheated samples 42, 43, 44, and 45 -- from 11 to 50.5 lesions per plant -- was probably due to the variation in plant reaction. The number of lesions produced on leaves of any two plants may vary, even though conditions of inoculation are the same. These samples, except for different moisture contents, were practically identical with check samples 46 and 47 which had 40 and 64 lesions, respectively.

In general, the results shown in Table 1 indicate a trend toward decreasing virus content with an increase in temperature, time, and moisture content.

The pH of the samples was included to show that there was a slight but regular reduction in pH as the time of exposure was increased. The high pH value of sample 45 was due to fermentation.

Samples 48, 49, and 50 revealed the presence of mosaic virus in stems from three other types of tobacco -- Burley, well-sweated Pennsylvania cigar filler, and flue-cured. These three samples represent run-of-factory material obtained from commercial stocks. They are included here to show the general widespread distribution of the virus. However, the amount shown for the Burley and Pennsylvania samples was slight. This may have been due to the age of the material, since both samples were from the 1948 crop. Autoclaved samples were included in the tests.

DISCUSSION

In considering the data in Table 1, a number of points should be kept in mind. The small number of lesions in most of the samples as compared with the number in the fresh green stem material (sample 52) was probably due to the following: (a) only about 60 percent of the plants used were infected; (b) the material was dry, a condition that inactivates some of the virus; (c) at a pH of about 7, tobacco mosaic is inactivated at a lower temperature than when the pH is about 5.5 (6).

For practical application, sterilization could probably be accomplished in a steam-jacketed rotating tube dryer, equipped with steam jets near the inlet to insure thorough moistening of the stems, and to heat them at 212° F. Since tobacco mosaic is soluble in a slightly alkaline pH, preliminary treatment with a weak solution of NaOH, KOH or lime may aid in virus extraction and expose more of the virus to the direct action of the heat. The stems may be dried toward the outlet end of the same machine, or in a separate dryer. They may then be packed for shipment in their "broken" condition, or ground for use as a fertilizer or conditioner. The usual precautions for grinding and handling the dried stems must be employed, since dry tobacco dust carries well-known fire and explosion hazards. An alternative method would be to grind the soft stem before placing them in the sterilizing equipment. This would greatly reduce the hazards of grinding and increase the rate of penetration of heat, but moist grinding would be more costly than dry grinding. A batch dryer suitably equipped for moistening and heating the stems, and then drying them, could also be employed.

Tobacco stems could also be treated in bulk in tanks or silos. Live steam could be supplied for sterilizing, after which the stems could be dried or disposed of in their soft, wet condition. The wet stems would be difficult to handle, however, and could not be economically transported any great distance.

SUMMARY

This exploratory study reveals that mosaic virus may be widely distributed throughout the tobacco-producing areas, and may be carried back to the land should unsterilized tobacco stems and waste leaf scraps be applied as a fertilizer or conditioner. The tests indicate that the virus in tobacco stems (32 percent moisture content) can be inactivated by exposure to a temperature of 212° F. in a saturated atmosphere for 15 minutes or longer.

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